Proc. Natl. Acad. Sci. USA
Vol. 73, No. 6, pp. 1897–1901, June 1976
Biochemistry

Solenoidal model for superstructure in chromatin

(electron microscopy/packing ratio/supercoil)

J. T. FINCH AND A. KLUG

MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England

Communicated by F. H. C. Crick, March 18, 1976

ABSTRACT **Chromatin prepared by brief digestion of nuclei with micrococcal nuclease, and extracted in 0.2 mM EDTA, appears in the electron microscope as filaments of about 100 Å diameter which coil loosely. In 0.2 mM Mg^{++} these "nucleofilaments" condense into a supercoil or solenoidal structure of pitch about 110 Å corresponding to the diameter of a nucleofilament. It is proposed that the x-ray reflections at orders of 110 Å observed in chromatin originate in the spacing between turns of the solenoid rather than that between nucleosomes along the nucleofilament. The solenoidal structure appears to need histone H1 for its stabilization. Under certain conditions, isolated nucleosomes can also aggregate into a similar structure. The solenoidal structure can be correlated with the "thread" of diameter about 300 Å observed by other workers in nuclei.**

The results of biochemical and other studies by various workers (1–4) led Kornberg to propose a model for the basic structure of chromatin (5). He suggested that it consists of a flexible chain of repeating structural units of about 100 Å diameter ("beads"), each containing a stretch of DNA 200 base pairs long condensed around a protein core made out of eight histone molecules (two each of the four main types). These elementary units have been named "nucleosomes" (6). The physicochemical parameters for the model require that the nucleosomes are in close contact along the chain, and there is now good evidence from electron microscopy that this is the case (7, 8). We call this close-packed chain a "nucleofilament." A recent study by low angle x-ray scattering of dilute solutions of chromatin, prepared without shearing, has given a mass per unit length consistent with nucleosomes densely packed along the length of a nucleofilament (9).

Linear arrangements of beads in a partly extended form ("beads-on-a-string") were first reported by Olins and Olins (10) and Woodcock (11) in chromatin spilling out of nuclei lysed on the specimen grid, and made visible by negative or positive staining. This extended form of chromatin probably results from the shearing forces present during the nuclear explosion. A similar extended appearance is seen in the electron micrographs obtained by Oudet *et al.* from extracted chromatin, after removal of histone H1 (6). However, electron micrographs of small oligomers of beads prepared from nuclei by brief nuclease digestion showed a preference for the nucleosomes to be in contact (8).

The nucleofilament can be correlated with the fairly uniform fibers or threads of 100 Å diameter seen in the electron microscope in chromatin specimens prepared by critical point drying in the presence of chelating agents (4). However, specimens prepared in the absence of chelating agents show thicker fibers of about 250 Å diameter (4, 12), suggesting that chromatin is normally present in a higher level of organization that breaks down when divalent ions are removed. The appearance of the thinner fibers has been attributed to the unwinding of tertiary coiling (13, 14) or to the separation of two associated 100 Å fibers (4, 14). Another line of reasoning, based on x-ray studies and described below, also suggests that Kornberg's linear chain model only represents the first level of folding in chromatin and that nucleofilaments are normally organized in a higher level of folding.

In this paper we present evidence obtained with the electron microscope for a state of chromatin in which nucleofilaments are folded into supercoils or solenoidal structures of pitch about 110 Å. In our experiments we have used chromatin prepared by a brief micrococcal nuclease digestion of nuclei, which is "native" in the sense that further nuclease digestion gives the same discrete pattern as does direct digestion of nuclei (15). Chromatin prepared by methods that involve shearing does not give such clear digestion patterns. In the presence of chelating agents, this native chromatin appears as fairly uniform filaments of 100 Å diameter, which are curly or loosely coiled. In the presence of Mg^{++} ions, tightly coiled forms of the nucleofilament are dominant, leading to a supercoil or solenoidal structure in which the pitch corresponds to the diameter of a nucleofilament.

MATERIALS AND METHODS

Chromatin was prepared from rat liver nuclei after brief micrococcal nuclease digestion, as described previously (15), to give an average length of about 40 nucleosomes, with a range roughly from 10 to 100.

Samples from dilute solutions of chromatin in appropriate media (e.g., 0.2 mM EDTA or 0.2 mM $MgCl_2$) were applied to carbon-coated grids and washed with the same medium. At the typical chromatin concentrations used, a solution containing 0.1 mM Mg^{++} is just sufficient to neutralize the phosphate groups. For negative staining, the grids were then washed with a few drops of 1% uranyl acetate, the excess being withdrawn onto a filter paper. For shadowing of freeze-etched specimens, the specimens were fixed by floating the support grid on a drop of 2% formaldehyde in the same solution for a few minutes and then were washed by floating on water. The specimens were then rapidly frozen in liquid nitrogen, dried on a Balzer's apparatus, and shadowed with platinum-carbon at an angle of 35°, as described by Nermut (16).

H1-depleted chromatin was prepared by treating chromatin prepared as above with 0.45 M or 0.6 M NaCl, and dialyzing against Tris buffer (5 mM Tris-acetate, 20 mM ammonium acetate, pH 6.8) after isolation on a sucrose gradient. Under these conditions, virtually all the histone H1 was removed with almost no concomitant loss of the other four histones (L. Lutter, unpublished work).

RESULTS

Electron microscope observations

Electron micrographs of chromatin in 0.2 mM EDTA or in 0.1 mM Mg^{++} are shown in Figs. 1 and 2. Chromatin appears as filaments about 100 Å in diameter, which often tend to coil up loosely. There is no visible repeat along the length of a filament. The filamentous character agrees with Sperling and Tardieu's

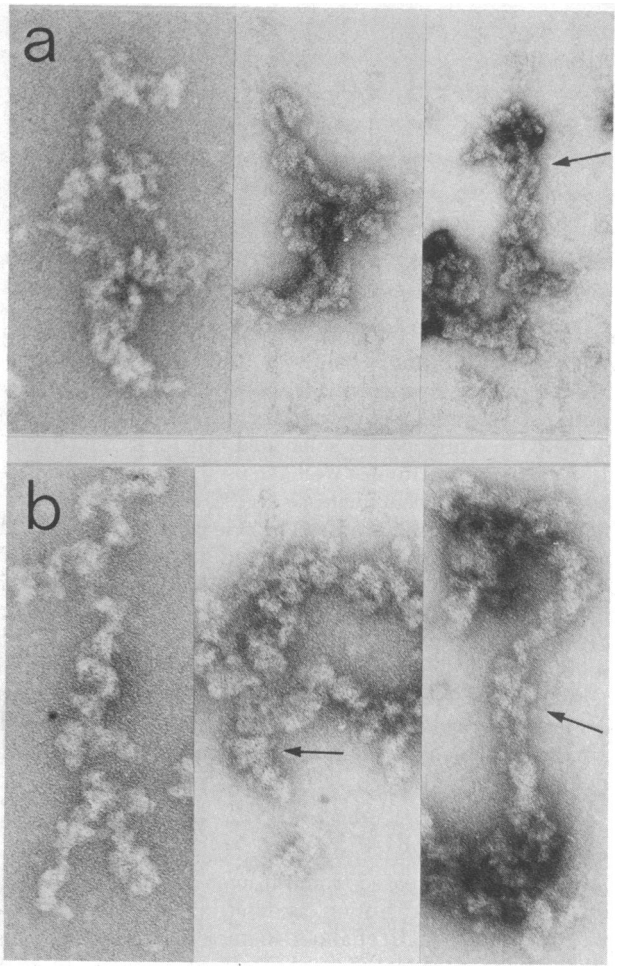

FIG. 1. Electron micrographs of negatively stained chromatin prepared in (a) 0.2 mM EDTA, or (b) 0.1 mM Mg^{++}. Arrows indicate regions where loose coiling is evident. ×140,000.

results on low angle x-ray scattering from dilute solutions of this material in the same medium, which are consistent with the scattering from a uniform cylinder of about 100 Å in diameter (9).

In a few instances (Fig. 1b) the coiling of the nucleofilaments is so pronounced as to lead to a more condensed state, but the majority of filaments are extended. In 0.2 mM $MgCl_2$, however, almost all the material has this condensed character. The concentration of Mg^{++} is not critical above 0.2 mM; similar results are obtained up to 1 mM Mg^{++}. In images of both negatively stained and shadowed specimens (Figs. 3 and 4) thick fibers or elongated particles can be seen, of a diameter that is somewhat variable, even along the same fiber, but is usually in the range 300–500 Å. In the negatively stained images striations can fairly often be seen running across the fibers, with a spacing of about 120–150 Å. We regard the smaller values as the more significant, since the longer spacings occur when the fibers are pulled out. The striations become increasingly difficult to detect as their spacing decreases, indicating the possibility of still tighter packing.

Similar striations can be seen in the shadowed specimens when the shadow direction is favorable, but there is little indication of further substructure in the transverse direction across the fiber width. These observations suggest that the fibers are formed by the winding up of the nucleofilaments into helices. The lengths of the shadows in Fig. 4 indicate that the fibers are

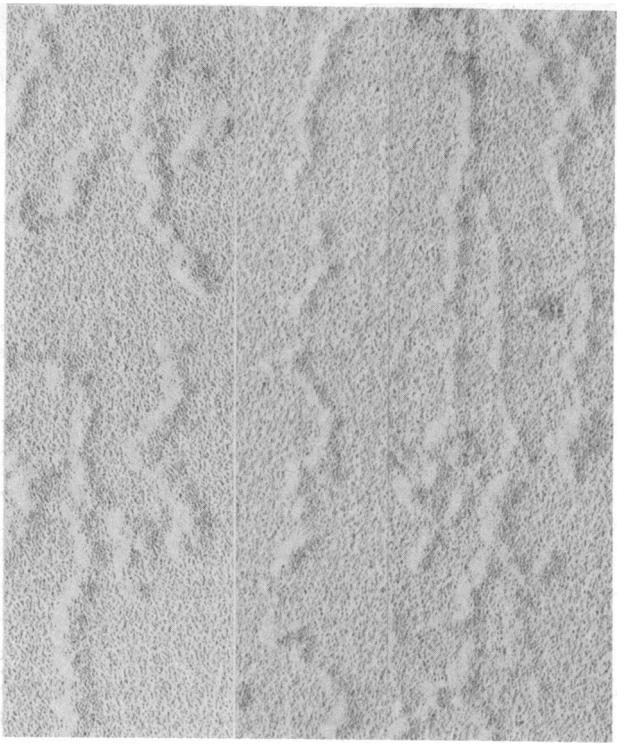

FIG. 2. Chromatin in 0.2 mM EDTA, freeze-etched and shadowed. The roughly parallel orientation of filaments probably arises during adsorption or washing of the specimen on the grid. ×140,000.

at least 300 Å thick, consistent with a helical character, rather than a two-dimensional zig-zag type of folding.

The direction of the transverse striations in shadowed specimens does not consistently indicate a right- or left-handed helix, although only one surface is being viewed. Since the slope of the helix is small, its left- or right-hand direction on one side of the fiber would be very susceptible to shear, and such shearing is quite often present, as judged by the changes in obliquity of the striations seen in both types of specimens. Indeed, the variations in diameter and occasional pulled out fibers show that the forces responsible for setting the relative phase of successive turns of the supercoil are not that strong or specific, at least for this type of preparation. Similarly, the helical parameters other than pitch are difficult to determine. The lack of visible detail along the turns of the solenoids and the variation in diameter make it difficult to judge the number of nucleosomes per turn, but the mean appears to be about 6 or 7. The variation in diameter may result from the relatively short length of the pieces of chromatin used (about 40 nucleosomes), which correspond to only six turns of helix on the average.

This tendency for an association involving the "sides" of a nucleofilament is also shown, under certain circumstances, by individual nucleosomes. Aggregates with an appearance similar to that of the Mg^{++}-induced fibers have been obtained from solutions of isolated nucleosomes (prepared as in ref. 17), which had been taken off a sucrose gradient and dialyzed against deionized water (Fig. 5). This suggests that this mode of aggregation is a natural one between nucleosomes and not dependent on the continuity of the DNA along a nucleofilament.

An electron micrograph of H1-depleted chromatin is shown in Fig. 6a. The particles no longer have the appearance of a continuous filament but rather of a chain of discrete beads, looking very like the micrographs published by Oudet *et al.* (6)

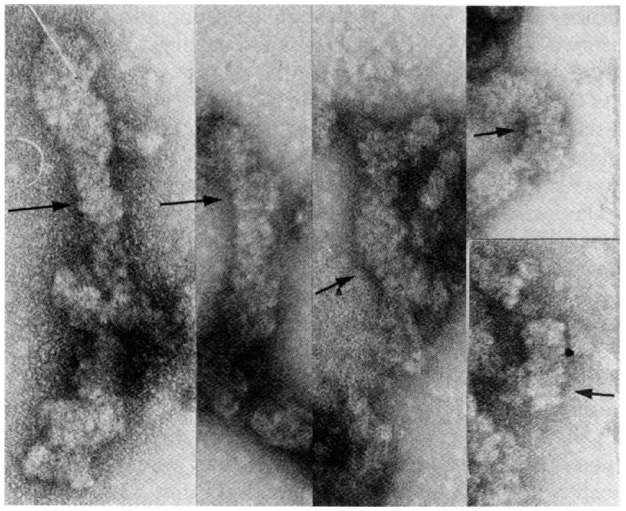

FIG. 3. Chromatin in 0.5 mM Mg^{++}, negatively stained. Arrows indicate transverse striations across elongated particles. ×140,000.

for H1-depleted chromatin. Addition of Mg^{++} (Fig. 6b) does not lead to condensed forms of the type described above: discrete beads are still evident, although the pieces of chromatin form more localized patches than without Mg^{++}. Similar results were obtained with chromatin depleted of H1 by use of tRNA (18). It is not clear whether removal of H1 produces a more extended form in solution, which will not condense into a supercoil, or whether its absence merely destabilizes the material on the grid of the electron microscope. In a very recent sedimentation analysis of pieces of chromatin up to 10 nucleosomes long, M. Noll and R. D. Kornberg (unpublished work) have found that removal of H1 causes a marked reduction in sedimentation constant, consistent with the opening out of a more compact structure. It would thus seem likely that H1 is needed for the formation or stabilization of the solenoidal structure.

Relation to x-ray diffraction studies

X-ray diffraction patterns of chromatin generally show a series of diffraction maxima or bands, nominally at 110, 55, 37, 27, and 22 Å, as originally described by Luzzati and Nicolaieff (19, 20) and by Wilkins *et al.* (3). The pattern becomes sharper and emerges from the background as the concentration is raised, becoming distinct at concentrations higher than about 35% (wt/wt). X-ray studies on fibers of chromatin have shown these maxima to be oriented in the fiber direction (21).

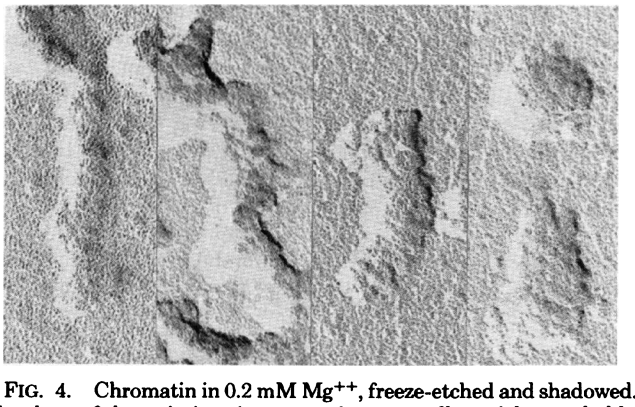

FIG. 4. Chromatin in 0.2 mM Mg^{++}, freeze-etched and shadowed. The slope of the striations is not consistent on all particles, probably because of distortion of the rather short particles, but there seems to be a preference for a left-handed helix. ×140,000.

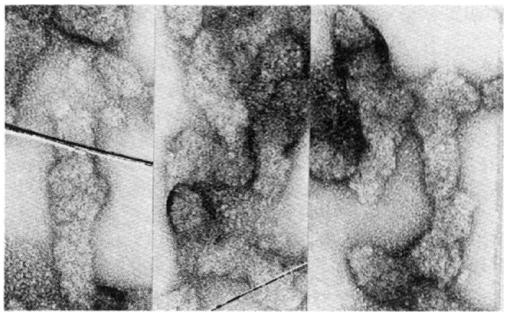

FIG. 5. Aggregates of nucleosomes in water, negatively stained. The nucleosomes were prepared and separated as described in ref. 17, and dialyzed for 24 hr against deionized water. The aggregates dissociate into individual nucleosomes on addition of 25 mM ammonium acetate, and are not found in the presence of 0.2 mM EDTA (8). ×140,000.

It would seem natural to associate this 110 Å x-ray spacing with the diameter of a nucleosome (5), and a discussion of its origin in terms of the packing of 110 Å spheres has recently been given (22). However, the 110 Å series of x-ray reflections cannot arise from the interval between nucleosomes along the nucleofilament direction, since the x-ray scattering curve in the region of spacings of 400–30 Å from nucleofilaments in dilute solution in 0.2 mM EDTA (9) fits the theoretical curve for a continuous density rod, with no signs of peaks at 110 Å or 55 Å. This is consistent with the lack of contrast seen along the nucleofilaments in the electron micrographs described above. Instead, we propose that the 110 Å spacing originates as a spacing between nucleofilaments organized in a higher level of folding. The simplest form of such a folding is a supercoil or solenoid with a pitch of 110 Å, and the electron micrographs shown above are evidence for a state of chromatin in which the nucleofilament is folded into such solenoidal structures. Our interpretation of the x-ray pattern is therefore that the 110 Å reflection and its higher orders arise from the spacing between the turns of a solenoidal type of structure.

Evidence for this interpretation has been provided by our colleague, L. Sperling, who has taken x-ray photographs of

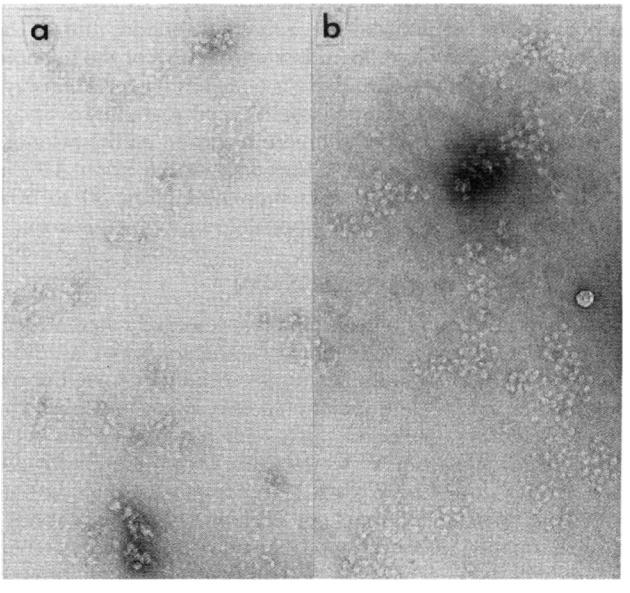

FIG. 6. (a) Negatively stained chromatin in Tris buffer, after depletion of histone H1 by salt treatment. (b) The same preparation in 1 mM Mg^{++}. ×75,000.

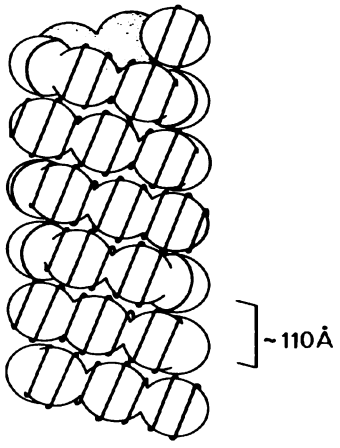

FIG. 7. Schematic diagram showing the folding of a nucleofilament into a solenoid. The thin line shown wound as a helix along the nucleofilament is intended to represent the folding of the DNA double helix on the outside of a protein core (5, 24, 8); it is highly schematic, since the path or fold is not known.

centrifuge pellets of material that showed solenoidal structures in the electron microscope. Although only at concentrations of 25% (wt/wt) or less, this material gave the characteristic type of x-ray pattern consisting of bands at orders of 110 Å; it showed more order than hitherto had been obtained, even at concentrations of 40% or over. This x-ray work will be described elsewhere (Sperling and Klug, in preparation), but briefly the character of the pattern is consistent with its arising from the turns of solenoids of low pitch angle. Indeed, our explanation for the x-ray pattern would be of the type originally proposed by Pardon and Wilkins, when they suggested their "supercoil" model for the DNA in chromatin (23). However, the structure we propose is at a higher level of folding, the path of the supercoil now being followed by the nucleofilament rather than the double helix of DNA.

We thus interpret earlier x-ray studies carried out without control of the Mg^{++} concentration as follows. The regular periodicity in the x-ray patterns and its variation with increasing concentrations of chromatin results from the formation of solenoidal structures on the withdrawal of water. At low concentrations the nucleofilament tends to be extended, and as the concentration is raised, more or less stable turns of the solenoid are formed. On this picture there is no necessity for a constant spacing between turns, and one might expect a gradual decrease in this spacing and also an increase in order, as the solenoid tightens. In fact, the x-ray spacings decrease by about 10% as the chromatin concentration is increased from 30 to 50% (wt/wt) (Kornberg, Klug, and Sperling, unpublished work).

DISCUSSION

The solenoidal form of chromatin is represented schematically in Fig. 7. For definiteness the nucleofilament is drawn as folded into a coil containing about six nucleosomes per turn, but our observations in the electron microscope do not establish this number. We have observed a fairly wide range of widths of the solenoidal particles, some of which variation is undoubtedly due to flattening, but would seem to represent a range of coils containing from 4 to 10 units per turn, with a mode at about 6 or 7. Presumably, under the conditions of our experiments there is a family of such solenoids all of nearly the same pitch (brought about by contacts involving the "sides" of nucleosomes) but with a varying number of units per turn. In most cases, the pitch angle is small, suggesting a single-start helix. However, a number are seen with a pitch angle large enough to correspond to a two-start helix, but as mentioned earlier, this appearance might arise by shearing of a simple helix.

Is there any evidence that the solenoidal structure actually exists in chromosomes, apart from the observations, referred to above, that "thick" (i.e., 250 Å diameter) fibers can be extracted from nuclei? The best evidence comes from the work of Davies and his colleagues, who have shown the presence of thread-like units in condensed interphase chromosomes or chromatin bodies. These tend to line up in layers at the surface of the nucleus, and the monolayers from a wide range of species have an average width of about 300 Å (25, 26). Previously it was supposed that threads roughly 170 Å in diameter were embedded in a matrix (27), but the latest interpretation of the staining characteristics (25) is that an inner rich DNA-core of this diameter is surrounded by an outer shell of external diameter 280 Å in which the protein-to-DNA ratio is higher. More recently it is suggested that the thread-like unit arises from some type of helical arrangement of the "beads-on-a-string" (28).

It would seem natural to correlate this "superunit" thread (25) with our solenoidal model, and to take a value of about 300 Å as an estimate for the outer diameter. Allowing for possibility of shrinkage in the specimens as prepared for the electron microscope, the solenoid in its native state would then have about six nucleosomes per turn of the helical coil. A bare nucleofilament consisting only of elementary nucleosomes wound into such a coil would leave a central hole of diameter about 100 Å. Thus, the solenoidal structure observed does not represent the closest packing of nucleosomes. The closest packing of spheres wound into a helix leaving no hole down the center would have about 2.7 units per turn (29). Presumably in chromosomes, the central hole in a solenoid might well be occupied by histone H1 and some of the nonhistone proteins or other control elements. It has been suggested to us by Dr. F. H. C. Crick that this central hole might also accommodate some of the "naked" DNA shown to exist as a small proportion of the total DNA in chromosomes after removal of histone H1 (30, 6). This would be in accord with Davies' suggestion of a DNA-rich core to explain the preference for uranyl-lead stain to pick out the centers of the threads, although this preference might also be accounted for by occlusion of the stain in a cylindrical cavity whose walls would necessarily be coated by the DNA wrapping around the nucleofilament.

The packing or compaction ratio for the DNA along the length of a densely packed nucleofilament is about 7:1 (5), and when the nucleofilament itself is wound into a solenoid of the type suggested, this packing ratio will increase to about 40:1 in the direction of the solenoid axis. Higher packing ratios can of course by achieved by coiling the solenoidal form of the nucleofilament into yet another order of helix, or by arranging a number of solenoids to wind around each other. It has been pointed out (31) that the construction of a hierarchy of helices can be achieved in a neat way by allowing the DNA double helix to kink locally rather than bend or distort over stretches, just as postulated for the folding of the DNA on the nucleosome itself.

We thank our colleagues Drs. R. D. Kornberg, M. Noll, L. Lutter, and especially Ms L. Sperling for supplying us with chromatin and for advice. We thank Dr. F. H. C. Crick for many helpful discussions.

1. Hewish, D. R. & Burgoyne, L. A. (1973) *Biochem. Biophys. Res. Commun.* **52**, 504–510.
2. Kornberg, R. D. & Thomas, J. O. (1974) *Science* **184**, 865–868.
3. Wilkins, M. H. F., Zubay, G. & Wilson, H. R. (1959) *J. Mol. Biol.*

1, 179–185.
4. Ris, H. & Kubai, D. F. (1970) *Annu. Rev. Genet.* **4**, 263–294.
5. Kornberg, R. D. (1974) *Science* **184**, 868–871.
6. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–300.
7. Griffith, J. D. (1975) *Science* **187**, 1202–1203.
8. Finch, J. T., Noll, M. & Kornberg, R. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3320–3322.
9. Sperling, L. & Tardieu, A. (1976) *FEBS Lett.* **64**, 89–91.
10. Olins, A. L. & Olins, D. E. (1974) *Science* **183**, 330–332.
11. Woodcock, C. L. F. (1973) *J. Cell Biol.* **59**, 368a.
12. Pooley, A. S., Pardon, J. F. & Richards, B. M. (1974) *J. Mol. Biol.* **85**, 533–549.
13. Dupraw, E. J. (1970) in *DNA and Chromosomes* (Holt, Rinehart & Winston, New York), Chap. 10.
14. Ris, H. (1974) in *The Structure and Function of Chromosomes*, CIBA Foundation Symposium 28 (Associated Scientific Publishers, Amsterdam), pp. 7–23.
15. Noll, M., Thomas, J. O. & Kornberg, R. D. (1975) *Science* **187**, 1203–1206.
16. Nermut, M. V. (1973) in *Freeze Etching*, eds. Benedetti, E. L. & Favard, P. (Societe Francaise de Microscopie Electronique, Paris), Chap. 12.
17. Noll, M. (1974) *Nature* **251**, 249–251.
18. Ilyin, Y. V., Varshavsky, A. V., Mickelsaar, U. N. & Georgiev, G. P. (1971) *Eur. J. Biochem.* **22**, 235.
19. Luzzati, V. & Nicolaieff, A. (1959) *J. Mol. Biol.* **1**, 127–133.
20. Luzzati, V. & Nicolaieff, A. (1963) *J. Mol. Biol.* **7**, 142–163.
21. Pardon, J. F., Richards, B. M. & Cotter, R. I. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 75–81.
22. Carlson, R. D. & Olins, D. E. (1976) *Nucleic Acid Res.* **3**, 89–100.
23. Pardon, J. F. & Wilkins, M. H. F. (1972) *J. Mol. Biol.* **68**, 115–124.
24. Noll, M. (1974) *Nucleic Acid Res.* **1**, 1573–1578.
25. Davies, H. G., Murray, A. B. & Walmsley, M. E. (1974) *J. Cell Sci.* **16**, 261–299.
26. Davies, H. G. & Haynes, M. E. (1975) *J. Cell Sci.* **17**, 263–285.
27. Davies, H. G. (1968) *J. Cell Sci.* **3**, 129–150.
28. Davies, H. G. & Haynes, M. E. (1976) *J. Cell Sci.*, in press.
29. Boerdijk, A. H. (1952) *Philips Res. Rep.* **7**, 303–313.
30. Varshavsky, A. J., Ilyin, Y. V. & Georgiev, G. P. (1974) *Nature* **250**, 602.
31. Crick, F. H. C. & Klug, A. (1975) *Nature* **255**, 530–533.